Citation:

Kurlandsky S, Stote K. Cardioprotective effects of chocolate and almond consumption in healthy women. *Nutrition Research*. 2006; 26(10): 509-516.

Study Design:

Randomized controlled parallel trial.

Class:

A - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

NEUTRAL: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

To look at the effects on selected cardiovascular disease (CVD) factors of combining chocolate and almonds as part of the Therapeutic Lifestyle Changes (TLC) diet in healthy individuals.

Inclusion Criteria:

- Healthy men and women age 22 to 65 years
- Serum total cholesterol between 4.1mmol per L and 7.8mmol per L (160mg per dL to 300mg per dL)
- No history of hypertension, artherosclerosis or metabolic disease
- No use of lipid-lowering medication or dietary supplements
- Willingness to maintain weight and adhere to usual exercise patterns throughout the study.

Exclusion Criteria:

- Known allergy to nuts or chocolate
- Smoking, alcohol intake greater than two ounces per day, and BMI more than 34kg/m^2.

Description of Study Protocol:

Recruitment

Community and campus-directed e-mail advertisements.

Design

Randomized four-armed, controlled parallel design.

Intervention

One of the following: 41g dark chocolate with a self-selected diet, 60g almonds with a self-selected diet, both the chocolate and almonds with a self-selected diet or a self-selected diet avoiding nuts and chocolate. The self-selected diet was based on the NCEP ATP III TLC diet.

Statistical Analysis

Univariate statistics were determined for the dependent variables. Repeated measures of analysis of variance were used to compare the characteristics of the four groups at baseline and changes in dietary intakes and biomarkers that occurred between baseline and end of treatment. Tukey post-hoc tests of comparisons were used for further analysis of differences among diet groups. Analysis included stratification of the sample population by baseline LDL cholesterol levels, subject age, and use of hormone replacement therapy (HRT).

Data Collection Summary:

Timing of Measurements

- Three-day diet records at baseline and two, four and six weeks
- Stanford Seven Day Physical Activity questionnaire at baseline and end of treatment
- Body weight at baseline and biweekly
- Blood samples at baseline and end of six-week treatment.

Dependent Variables

- *Diet intake:* Three-day food records on two weekdays and one weekend day. Completeness and accuracy was checked by a registered dietitian (RD). Records were analyzed for total energy, total fat, SFA, MUFA, PUFA, cholesterol, carbohydrate and protein, total and soluble fiber and selected vitamins and minerals using Food Processor SQL software, version 9.2.
- *Physical activity:* Stanford Seven Day Physical Activity questionnaire
- *Body weight:* Digital scale
- *Serum lipids:* LDL, HDL, total cholesterol and triacyglycerols after a 12-hour fast at a commercial clinical lab using methods according to the Standardization Program of CDC and NHLBI. LDL was calculated using Friedewald formula.
- *Inflammatory markers:* Colorimetric enzyme-linked immunosorbant assay using commercially available kits.

Control Variables

Diet education from an RD based on guidelines and materials provided by NCEP ATP III to include total fat of 25% to 35% of calories from fat, less than 7% SFA, up to 10% PUFA and up to 20% MUFA. Dark chocolate was provided as a candy bar (Dove Silky Dark Chocolate, 1.3oz). All nuts other than almonds, and other chocolate, cocoa, and chocolate-containing foods were to be eliminated. Foods high in flavonoids [red wine, green and black tea, fruit (especially berries)] and selected vegetables were to be limited.

Description of Actual Data Sample:

- *Initial N:* 52 women
- *Attrition (final N):* 47
- *Age:* 43.7 years for all groups. After randomization, chocolate group had a mean age of 36.5 years.
- *Other relevant demographics:* Two subjects (one in control and one in chocolate and almond group) took HRT

- *Anthropometrics:* Physical activity levels were similar at baseline. Activity, weight and BMI remained stable throughout the study
- *Location:* Syracuse University and State University of New York Upstate Medical University.

Summary of Results:

Variables	Chocolate Group Measures and Confidence Intervals	Almond Group Measures and Confidence Intervals	Chocolate and Almond Group Measures and Confidence Intervals	Control Group Measures and Confidence Intervals	Statistical Significance of Group Difference
Total Cholesterol (mmol per L)	0.08±0.21	0.01±0.10	-0.04±0.12	-0.01±0.21	0.22
LDL cholesterol (mmol per L)	0.14±0.15	0.02±0.10	0.11±0.16	0.09±0.15	0.16
HDL cholesterol (mmol per L)	0.04±0.08	-0.01±0.05	-0.03±0.09	-0.05±0.08	0.50
Triacyglycerols (mmol per L)	-0.22±0.08	-0.21±0.22	-0.23±0.06	-0.12±0.07	0.08
ICAM (ng per mL)	-19.2±14.5	-2.6±29.3	-2.44±21.0	3.7±38.1	0.49
VCAM (ng per mL)	-43.4±7.1	-52.2±23.6	-44.2±26.4	-68.1±40.5	0.80
hsCRP (mg per L)	1.1±1.3	-1.8±1.4	-1.2±2.1	-1.3±1.2	0.60

- ICAM: Intercellular adhesion molecule
- VCAM: Vascular adhesion molecule
- hsCRP: High-sensitivity C-reactive protein.

Other Findings

There were no significant differences in baseline diet intakes. There were statistically significant differences among diet groups for energy; percentages of total fat, SFA, PUFA, and MUFA; cholesterol; total fiber; percentages of energy from protein and carbohydrate and vitamin E.

Consumption of study products accounted for most of the observed dietary differences among groups, particularly in the two groups receiving almonds. Total energy and fat intake were higher in all of the treatment groups than in the control group. The percent of energy from total fat increased in the almond and almond and chocolate groups and decreased in the control group. The percentage of calories from SFA decreased across all groups except the chocolate-only group. The percentage of calories from the PUFA and MUFA plus dietary fiber and vitamin E increased in both almond groups. Dietary cholesterol decreased in all groups from baseline to the end of

treatment to below the recommended 200mg. Percent energy from SFA remained slightly higher than recommended.

98% of subjects assigned to the treatment groups consumed all assigned study product.

Author Conclusion:

Consumption of chocolate and almonds as part of the TLC diet for six weeks led to favorable dietary changes and showed no harmful effects on subjects' weights, serum lipids and inflammatory markers. A favorable effect of chocolate consumption on circulating ICAM levels and observed improvements in serum triacylglycerols warrant investigation with a larger sample size.

Reviewer Comments:

Significance of differences for diet between the groups was difficult to determine from Table 3.

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions		
1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	Yes
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes

Validity Questions		
1.	**Was the research question clearly stated?**	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes
2.	**Was the selection of study subjects/patients free from bias?**	Yes

2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes
2.2.	Were criteria applied equally to all study groups?	Yes
2.3.	Were health, demographics, and other characteristics of subjects described?	Yes
2.4.	Were the subjects/patients a representative sample of the relevant population?	Yes
3.	**Were study groups comparable?**	No
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	No
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	No
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	Yes
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	N/A
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
4.	**Was method of handling withdrawals described?**	Yes
4.1.	Were follow-up methods described and the same for all groups?	Yes
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	Yes
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	Yes
4.4.	Were reasons for withdrawals similar across groups?	Yes
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
5.	**Was blinding used to prevent introduction of bias?**	No

5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	No
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	No
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	N/A
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**	Yes
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	Yes
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	N/A
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	Yes
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	Yes
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	N/A
6.6.	Were extra or unplanned treatments described?	N/A
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A
7.	**Were outcomes clearly defined and the measurements valid and reliable?**	Yes
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	Yes
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
7.6.	Were other factors accounted for (measured) that could affect outcomes?	Yes

	7.7.	Were the measurements conducted consistently across groups?	Yes
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**		N/A
	8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes
	8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
	8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
	8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	N/A
	8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	N/A
	8.6.	Was clinical significance as well as statistical significance reported?	N/A
	8.7.	If negative findings, was a power calculation reported to address type 2 error?	N/A
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**		Yes
	9.1.	Is there a discussion of findings?	Yes
	9.2.	Are biases and study limitations identified and discussed?	Yes
10.	**Is bias due to study's funding or sponsorship unlikely?**		???
	10.1.	Were sources of funding and investigators' affiliations described?	Yes
	10.2.	Was the study free from apparent conflict of interest?	???